Cover Page

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EFFECT OF DIETARY SELENIUM NANOPARTICLES (SeNPs) ON GROWTH, HEMATOLOGY, PROTEIN PROFILE, IMMUNE RESPONSE, AND *E. coli* BACTERIA CHALLENGED ON ROHU, *Labeo rohita*

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Abstract

The aim of the present study was synthesis of selenium nanoparticles (SeNPs) employing reducing agent of ascorbic acid methods and synthesized particles was characterized through UV–vis, SEM, EDX, zeta potential measurements. The 60 days supplementation feeding trail was conducted to evaluate the effects of diet fed with Sodium Selenite (Se) and Selenium Nanoparticles (SeNPs) on growth, hematology, protein profile and immunological activity in rohu, *Labeo rohita*. Basal diets integrated with 0.5mg/kg^{-1} of Sodium Selenite (Se) and Synthesized Selenium Nanoparticles (SeNPs) on fed with fish feeds. Enhancement of growth performance, hematology, protein profile and survival rates were noticeable in SeNPs compared to Se and C groups. Likewise, bacterial challenged replicates were observed in Se and C groups that appear virtually average by using SeNPs. Therefore, finally SeNPs 0.5 mg/kg−1 stimulate impact on *Labeo rohita* where the same dose from SeNPs was more multi potent acting as immunomodulating against *E.coli* pathogens.

Keywords: Nanoparticles, *E. coli*, Aquaculture, Growth Performance, Immune Response.

Introduction

Nanotechnology is an emergent technology with high potential use for various application in the aquaculture industry [1]. Nano minerals (<100 nm) are characterized by superior surface area affinity, higher solubility, low toxicity, sustained release, and functionality well recognized in aqua feed [2], [3]. Selenium is a significant dietary micronutrient [4] necessary for the regulate body functions and metabolism of animals [5]. Selenium (Se) particles are one of the microelements involved in various tasks in the entire body of aquatic animals [1], [6]. Selenium nanoparticles are crucial trace minerals, which involved in assorted tasks in the whole body of aquatic organisms, with high efficient to regulate as a growth promotor, antioxidant, and immunostimulant agent in aquaculture industries [7, 8]. Moreover, it has been potential to act as antiviral, antifungal, and antibacterial activity [9]. Furthermore, nutritionally balanced aqua diets is the important key aspect that helps neutralize these stressors foremost to high output and well-being [10]. Recently, Selenium microelements are fundamental tactic to assurance the balance of the nutritional value of aqua diets.

Labeo rohita (Rohu) is one of the majorities of cultivable carps in India, with higher consumer predilection due to their fast growth and high excellence flesh. Currently, new aquaculture systems are well treated with inorganic uses of chemicals and fertilizers for short time production. The farmers are exceeding stocking in the aquaculture ponds and providing artificial feeds for faster growth of the farmed fish. All though, a variety of pathogenic bacteria infections are causing through these inventive practices in fish species, which cause vast mortality in farms and hatcheries [11],[12],[13].

Fish pathogens are mostly obsessed using antibiotics. But, constant severe usage or misuses of antibiotics may lead to the progress of reproving drug resistance that vitiated the inefficiency of drugs [14]. *Escherichia coli,* usually accompanying to fish consumption abscessed by those bacterial species especially infects the intestine and causes several symptoms these can include- abdominal pain, diarrhoea, bright red bloody stools, nausea, in some cases fever, fatigue in humans [15],[16]. All year the fish and fishery trade good by the importing countries due to existence of *E. coli,* cause high commercial losses. The present study aimed to evaluate the effect of dietary SeNPs on improves the physio-biochemical and immunological health status of rohu, *Labeo rohita*. This study is very useful for aquaculture hatchery to eradicate the harmful diseases and increase the fish production.

2. Materials and Methods

2.1. Materials

All the chemicals were purchased from Hi-media Pvt., Ltd

2.2. Synthesis and Characterization of Selenium nanoparticles

To synthesis of selenium nanoparticles (SeNPs) stock solution preparation: 5mM of Na_2SeO_3, 20mM ascorbic acid was dissolved in 10ml of Mili-Q water and keep them separately. Prepared ascorbic acid solution was added gently drop wise into the

Cover Page

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stirred sodium selenite solution at25˚C for 12hrs. After the addition of the ascorbic acid solution, the colour changes were observed at brick red colour precipitate formed. Then, the precipitate was centrifuged for 15min at 8000rpm.The precipitate were several time washes with the distilled water for removing waste materials. Finally, the particles were dried and backed with air tight bags. Then, maintained for room temperature, until the use of further experimental studies [17]. Furthermore, microscopic and spectroscopic techniques were employed to characterize the synthesized SeNPs. These techniques are: UV-vis spectroscopy, Scanning electron microscope, E Dax and Zetapotencial.

2.3. Fish Collections and Experimental design

The 500*Labeo rohita* fingerlings (3.43 ± 0.41g) were purchased from the Tamil Nadu Fish Hatchery, Alayar. These fishes were acclimatization for 10 days and feed with rice bran for maintenance (Table 1). Thenceforth, At the density of 20 experimental animals were indiscriminately distributed into five poly vinyl circulating troughs tank. Fishes were fed with a twice a day [18]. In this study, Fishes were separated into two different trail groups, and each groups maintained three replicates.

First trail groups were checking the feeding efficiency. The control group (C) was fed on a basal diet, second group was basal diets fed with sodium selenite (Se-0.5 mg/Kg^{-1}) and third groups were basal diets fed with selenium nanoparticles (SeNPs-Se-0.5 mg/Kg^{-1}). The feeding trails were maintained at 60 days. The uneaten feed was collected after active feeding approximately for 40 min with the help of siphoning pipe tubes.

The collected feed was then oven-dried at 100˚C to calculate the final feed conversion ratio (FCR). No feed was offered to the fish on the day of weekly measurement. At the end of the experimental trail, desired numbers of fish were randomly sacrificed for the assessment of whole-body composition.

Table 1: Ingredients and Chemical composition of the experimental diets (g/kg)

Ingredients	**Grams (g)**
Fish meal	30
Soybean meal	20
Rice bran	20
Egg albumin	3
Cod liver oil	3
Wheat flour	5
Topioca flour	5
Groundnut Oil	10
Vitamins & mineral mixture	4
Total	**100**
Composition	%
Crude Protein	27.69
Moisture contents	9.60
Crude fiber	2.18
Ash	6.98

2.4. Growth performance

Eight fishes were randomly selected from each experimental groups and then measured Total weight gain, Specific growth rate, and feed conversion ratio were calculated [19].

Various parameters as follows:

- Total weight gain (g $fish^{-1}$) = WT-WI, whereas WT is a final weight, WI is an initial weight
- The specific growth rate (SGR-% day^{-1}) = 100× (In WT-In WT)/duration/day
- The feed conversion ratio (FCR) = total feed intake (g)/total gain (g).
- Survival percentage = Number of fish in each group remaining after the 60 days period/ initial number of fish × 100

Cover Page

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2.5. Hematology

After finished 60 days feeding trail, the blood was drawn from the caudal vein of randomly selected fishes and then blood rinsed with EDTA (2mg⁄ml) an anti-coagulant. The estimation of hemoglobin was followed by using Sahli's haemoglobinometer [20]. Erythrocytes (RBC) indices in the blood were ascertained reported [21]. White blood cells were resolute using a Neubauer haemocytometer as delineate by Kaplow [22].

2.6. Total tissue protein content

For the determination of total protein content in liver and muscle tissues was estimated by employing the Folin- Ciocalteau method of Lowry [23].

2.7. Challenge test

Bacteria strain of *E. coli* (MTCC-443) was purchased from Microbial Type Culture Collection, Pune.The bacteria culture was maintained in Muller Hinton Broth (MHB) at 37˚Cfor 24 hours. After finished experimental trail fishes were separately into two replicates from each groups and then intraperitoneally injected with the (MTCC 443- *E.coli*) at concentration of (1.2×10^8 CFU/ml) [24]. All fish were kept under watching for 2 weeks to attainment the daily noticed abnormal clinical signs

2.8. Lysozyme activity

Lysozyme activity (µg mL^{-1}) in blood serum was dictated by a method represented by [25]. Collected blood samples were centrifuged at 1500rpm for 15min. After that, serum was separated and stored –20 °C in freezer. The 900 µL of a 0.75 mg mL^{-1} *Micrococcus lysodeikticus* (Sigma Aldrich, India) suspension (in phosphate buffered saline; pH 6.2) added into the 100 µL of serum containing test tubes and shaken well.

2.9. Statistical analysis

All analyses were used in one-way analysis of variance by SPSS software package 16.0 version.A probability level of $P<0.05$ was used to test significance of differences among values.

3. Results and Discussion

3.1. Synthesis and Characterization of Selenium nanoparticles

3.1.1. UV–Visible Spectroscopy

Produced selenium nanoparticles have been evaluated by different methods. At UV/vis analysis in 200-500nm, the absorption peak was obtained at 271nm that is related to selenium nanoparticle **(Fig.1).** The Maximum absorption peak at 274 nm can be attributed to the size of SeNPs and the decline in absorption peak suggests the aggregation of synthesized nanoparticles [26].

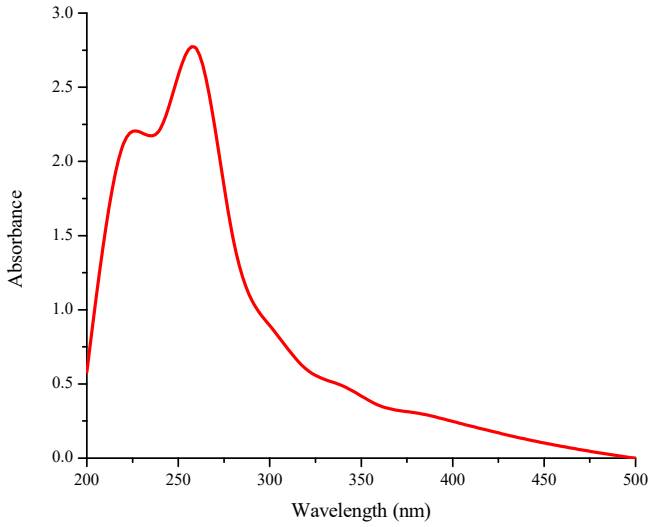

Fig.1. UV–vis spectra of aqueous sodium selenite with ascorbic acid

Cover Page

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3.1.2. Morphology and elemental studies

The morphological features of the SeNPs were measured using SEM. Particle morphology shape and size with SEM Microscope reveals that rods shape with the size of the particle was in the range 60 to 70 nm for SeNPs **(Fig.2).** Recently, reported that selenium nanoparticles showed a morphological shape of rods-shape with size of the nanoparticles range in 74nm which evident the natural character of selenium [27].

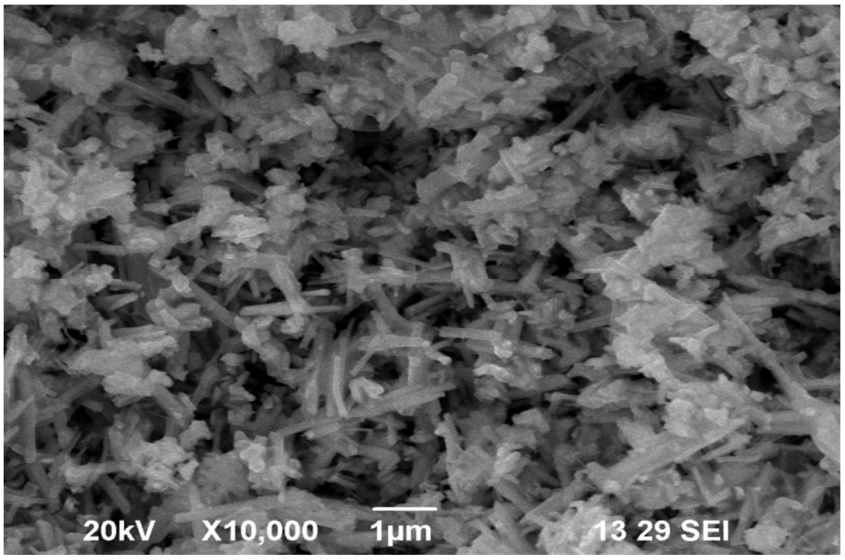

Fig.1. Scanning electron microscopy of synthesized selenium nanoparticles

Further, chemical composition of the synthesized selenium nanoparticles was also confirmed by EDAX. The EDAX spectrum of the nanoparticles, shown in **Figure 3**, also indicates that Selenium is the major compound present in nanoparticles. The higher amount of selenium present in the spectrum confirmed the prepared Nano-selenium suspension [28].

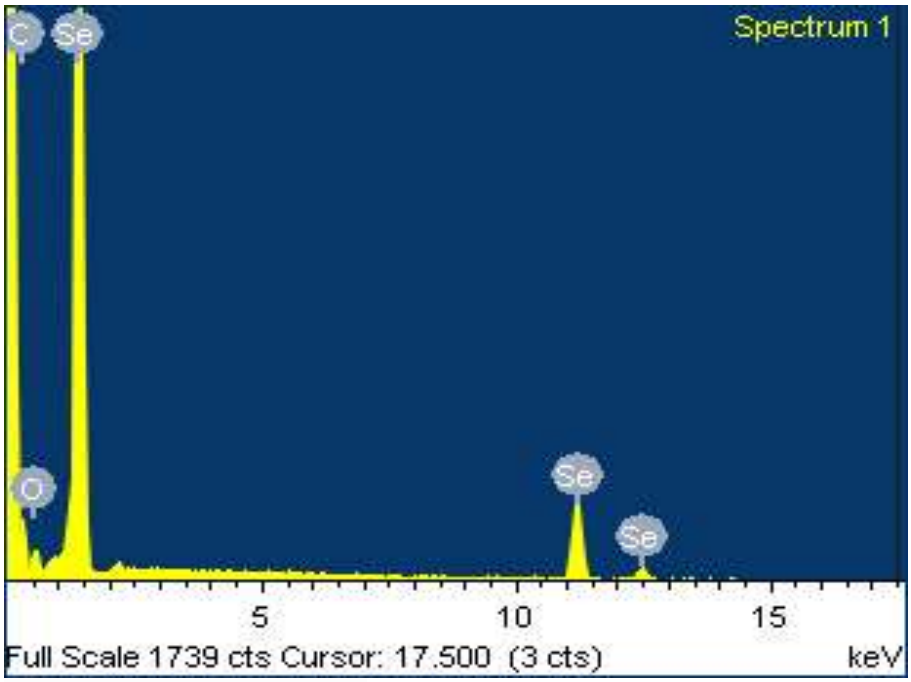

Fig.3. Energy dispersive X-ray spectrum of synthesized selenium nanoparticles

Cover Page

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3.1.3. Zeta potential measurements

Zeta potential (ZP) observed a value of -25 mV for chemical composition of the synthesized selenium nanoparticles showed that it is constant due to the electrostatic repulsive force **(Fig 4 and Table 2).** In addition, The zeta potential value recorded that the synthesized SeNPs was −24.01 mV which indicated that the synthesized nanoparticles had high stability which is in agreement with previous reports [29].

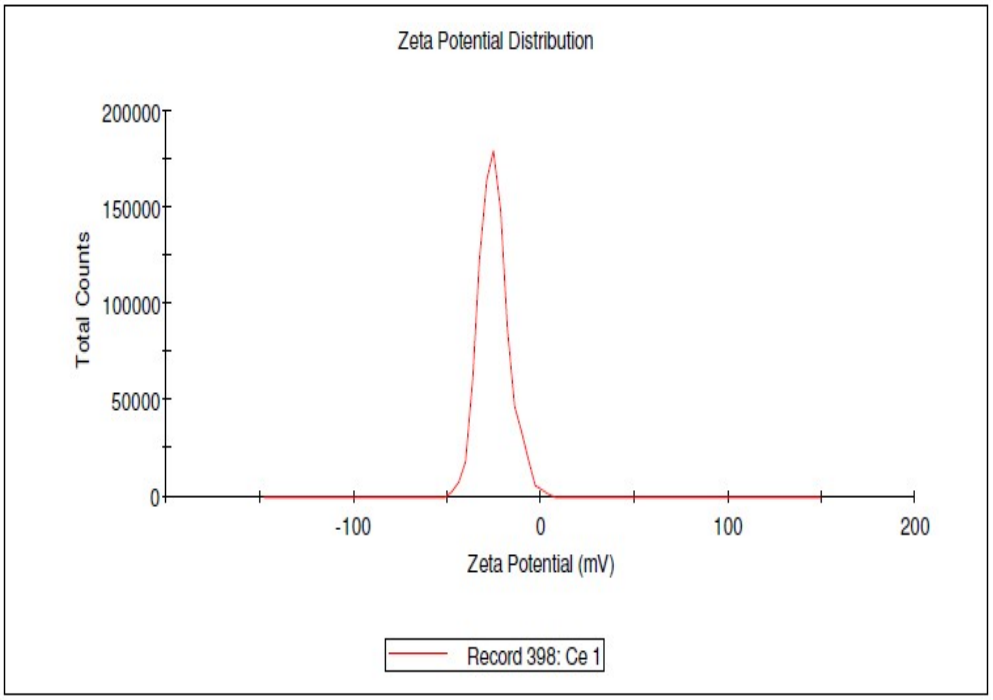

Fig. 4. Zeta Potential of synthesized selenium nanoparticles

Table 2: Zeta Potential results of synthesized selenium nanoparticles

Results	**Mean (mV)**	**Area (%)**	**Width (mV)**
Zeta potential (mV): -25.1	Peak 1: -25.1	100.0	8.07
Zeta deviation (mV): 8.07	Peak 1: 0.0	0.0	0.00
Conductivity (mS/cm): 1.54	Peak 1: 0.0	0.0	0.00

3.2. Growth Performance

The detailed growth parameters measurements of *L. rohita* fed with Sodium Selenite (Se) and Selenium nanoparticles (SeNPs) diet are represented in **Table 3**. such as weight gain (WG %), final body weight (FBW (%), SGR (%) and Survival Rate (SR %) were significantly higher ($P < 0.05$) in SeNPs group compared then Control (C) group. FCR of *L. rohita* that fed the diet contain SeNPs was significantly lower ($P < 0.05$) than that fed with Se and Control diets. Moreover, earlier reported that *Nile tilapia* fish nano-Se supplemented diets showed significantly higher on FBW, BW gain and SGR with significantly lower at FCR [30]. This finding shows that the SeNPs enhance the intestinal protein contents in epithelial cells turn onto healthy metabolism of SeNPs feed consumption to subsequent in higher growth [31].

Table 3: Growth performance of rohu fish, *Labeo rohita* (means ± SE) fed on diets containing Se, SeNPs for 60 days

Parameters	**Controls (C)**	**Sodium Selenite (Se)**	**Selenium Nanoparticles (SeNPs)**
Initial Body Weight(g)	3.43 ± 0.41^{c}	3.5 ± 0.39^{bc}	3.7 ± 0.39^{a}
Final Body Weight(g)	5.8 ± 0.61^{c}	8.9 ± 0.29^{b}	9.8 ± 0.84^{a}
WG	6.4 ± 0.62^{c}	7.3 ± 0.32^{b}	8.3 ± 0.62^{a}
FCR	3.63 ± 0.12^{a}	2.86 ± 0.05^{b}	1.83 ± 0.24^{c}

Cover Page

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SGR	1.63± 0.18^{c}	2.81± 0.15^{b}	3.96± 0.41^{a}
Survival Rate%	81.5± 1.06^{c}	91.05±0.84^{b}	99.67±0.46^{a}

Data are delineated as mean ± SE (n = 30). Means ensue by a different letter within a column are significantly different ($P < 0.05$)

3.3. Total protein content

The SeNPs **(Table 4)** supplemented diets were observed significant increased ($P < 0.05$) the protein profile in the muscle and liver tissues of *L. rohita* whereas compared to the control diets. Similarly, Selenium is one of the important to improve the protein content in fishes and vertebrates [32]. Selenium was released selenoprotein shows the in good health biological activity [33].

Table 4: Effects of dietary SeNPs on total protein content in muscle and liver tissues of juvenile *L. rohita* after 60 days of supplementation.

Parameters/diets	Total protein content (mg/g^{-1})	
	Muscle	Liver
Control	7.03± 0.12^{a}	6.66± 0.26^{b}
Sodium Selenite (Se)	20.9± 1.86^{a}	15.13± 0.16^{b}
Selenium Nanoparticles (SeNPs)	28.74± 1.31^{a}	19.34± 1.94^{b}

Data are delineated as mean ± SE (n = 30). Means ensue by a different letter within a column are significantly different ($P < 0.05$)

3.4. Hematology Parameters

The hematological profile of *L. rohita* with Selenium nanoparticles (SeNPs) diet as exposed in non-challenged and bacterial challenged are represented in **Table 5**. Here, non-challenged groups were observed significant ($P < 0.05$) increase at RBCs, Hb, and Hct % in SeNPs supplemented diets while compare than others. Whereas, the bacterial challenged groups reveal significant recession in RBCs count and Hb level in control and while the SeNPs group remain unaffected. Similarly, several research studies have also confirmed the role of selenium in improving the hematological indices of fishes [34], [35]. However, the non-challenged WBC parameters was noticed significant ($P < 0.05$) decreased at SeNPs groups compared to control. However, Bacteria challenged groups were noticed significant ($P < 0.05$) raised WBC count in SeNPs groups compared to control. Similarly, several research previously studies have unchanged SeNPs in shielding erythrocyte from hemolysis either by prominent antioxidant impact [36], [37] and antimicrobial effectiveness neutralize infection [1].

3.5. Lysozyme activity

The lysozyme activity was significant increased ($P < 0.05$) at non- challenged groups in SeNPs compared Se and Control groups are represented in **Table 5**. At the same time, Bacterial challenged groups were observed decrease lysozyme activity in Se and Control groups, but SeNPs lysozyme activity remain unchanged. Previously, reported that the lysozyme activity Selenium nanoparticle supplementation definitely superior the innate immunity by improving the serum lysozyme activity [38] and keeps fish in ideal [39].

Table 5: Hematological parameters of rohu fish, *Labeo rohita* (means ± SE) fed variable forms of dietary SeNPs for 60 days and post-challenge with *E. Coli*

Parameters	Non-challenged			Bacteria Challenged		
	C	Se	SeNPs	C	Se	SeNPs
RBC (10^{6}/ml)	1.35± 0.08^{bc}	1.29± 0.08^{d}	1.43± 0.04^{a}	0.94± 0.11^{d}	1.13±0.03^{bc}	1.38± 0.01^{a}
Hb (g/dl)	8.43± 0.40^{d}	8.76± 0.40^{bc}	9.06± 0.12^{a}	6.46 ±0.30^{d}	7.7± 0.92^{bc}	8.16± 0.74^{a}
Hct	28.09± 2.16^{d}	33.09±0.70^{bc}	36.37± 2.62^{a}	24.13± 0.72^{d}	29.4±0.46^{bc}	30.81± 1.0^{a}
WBC	18.9± 0.29^{a}	17.91± 0.30^{bc}	14.5± 0.28^{d}	15.3± 0.29^{d}	18.1±0.09^{bc}	19.7± 0.16^{a}
Lysozyme activity (µg mL-1)	1.33±0.42^{d}	1.7±0.28^{bc}	2.00±0.08^{a}	0.90±0.01^{d}	1.00±0.13^{bc}	1.94±0.42^{a}

Evaluates with dissimilar superscript capital differ significantly at $p < 0.05$, C= Control, Se= sodium selenite, SeNPs = selenium nanoparticles, RBCs=erythrocytes, Hb=hemoglobin, Ht=hematocrit, WBC=white blood cells.

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4. Conclusions

From the early results, we can resolve that using of SeNPs 0.5mg/ kg^{-1}induce Growth parameters, Protein profile using muscles and liver tissues, Hematological and immunosuppressive between non-challenged and bacterial challenged significant impacts on *L. rohita* fishes.

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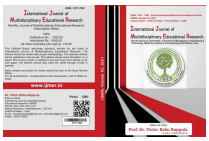
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